MEASUREMENT OF CORNEAL OXYGEN CONSUMPTION AND HEXOSE MONOPHOSPHATE SHUNT ACTIVITY IN MARINE FISH AND BUPHTHALMIC RABBITS

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Corneal transparency in various species is the result of a finite structural development, the possession of limiting tissue barriers (epithelium and endothelium) and the continued maintenance of its basal metabolism. Because of its location, the cornea obtains its oxygen for aerobic metabolism directly from the atmosphere (mammalian species) and the environmental water (aquatic species). The cornea derives its energy from the metabolism of glucose, which is primarily metabolized by the glycolytic pathway. Of all glucose consumed 88 percent is metabolized no further than lactate, leaving 12 percent to be oxidized by its cellular respiratory systems (Riley, Exp. Eye Res. 8:193, 1969). Past studies have also shown that the epithelium can metabolize glucose through the hexose monophosphate shunt (HMS) (Kinoshita and Masurat, Am. J. Ophth. 48:47, 1959) and more recently we have shown that the HMS is present and can be activated in the corneal endothelium.

The buphthalmic rabbit is characterized phenotypically by an eye with an increased intraocular pressure (IOP) and is of considerable interest because of the similarity of its ocular pathology to congenital glaucoma in man. The corneas of these rabbits are subject to the continued stress of an elevated IOP and eventually become opaque. Since corneal transparency is largely a function of the tissues metabolic status, the determination of the metabolic state of these compromised corneas is of considerable interest.

The present studies were undertaken (1) to measure basal corneal oxygen consumption (QO_2) in marine teleosts and elasmobranchs in order to compare these values to previous studies on fresh water (FW) teleosts and mammalian corneas, (2) to determine HMS activity in the scuplin cornea, a tissue under extreme osmotic stress, and (3) to evaluate HMS activity in the component layers of the buphthalmic rabbit cornea.

Corneas were excised from sculpin, *Myoxocephalus scorpius*; winter flounder, *Pseudopheuronectes americanus*; goosefish, *Lophius americanus*, and the spiny dogfish, *Squalus acanthias* for measurement of QO_2, % H_2O and Q_{10}. The in vitro QO_2's were made with a YSI biological oxygen monitor, Clark-type O_2 electrode and a chamber modified specifically for QO_2 measurements of excised corneas (Edelhauser, Exp. Eye Res. 19:317, 1974). Corneas of the marine teleosts were placed in 2 cc of Forster's Flounder Medium (Sci. 108:65, 1948) with 16 mM HCO_3. For the dogfish, Forster's Elasmobranch Ringer's (Comp. Biochem. Physiol. 43A:3, 1972) was used. Following a 20 min. QO_2 determination, the corneas were removed from the chamber, blotted, weighed and dried at 105°C for 24 hrs to constant dry wt.

To determine HMS activity in each of the corneal layers of the sculpin, three groups of corneal tissue, whole corneas, outer and inner corneas (Fischer and Zadunaisky, Exp. Eye Res. 25:149, 1977) were incubated at 15°C in the presence of selectively labeled (.25 µCi of ^{14}C-1 or ^{14}C-6) glucose.

Corneas from the buphthalmic rabbits were obtained from the Jackson Laboratory and divided into three groups: (1) an epithelial scraping, (2) an endothelial sheet and (3) the remaining stroma. The corneal tissue was placed in 10 ml Ehrlenmeyer flasks containing 3.2 ml bicarbonate Ringers plus glucose (90 g/l). The HCO_3 Ringers was composed of NaCl (6.51 g/l), KCl (0.359 g/l), $CaCl_2$ (0.115 g/l), $MgCl_2$ (0.159 g/l) NaH_2PO_4 (0.103 g/11) and $NaHCO_3$ (2.454 g/l). The rate of $^{14}CO_2$ evolution at 37°C with selectively labeled (^{14}C-1 or ^{14}C-6) glucose as a substrate was similarly used to investigate the role of the HMS in these corneal tissues.

The results of the QO_2 measurements are listed in Table 1. The QO_2's for all the marine species studied are similar, but considerably lower than those measured for the FW trout and rabbit corneas (Exp. Eye Res. 19:317, 1974). This may reflect the large number of total corneal cells that are present in both

Table 1

Mean Values (± SE) of Marine Teleost and Dogfish Corneal Oxygen Consumption, Hydration and Q_{10} Values

	oxygen consumption $\mu l\ O_2\ mg^{-1}$ dry wt hr^{-1}		hydration % H_2O	Q_{10}
	14.5°C	20°C		
Sculpin	0.358 ± 0.033 (4)	0.446 ± 0.014 (4)	85.56 ± 0.35 (10)	1.48
Flounder	0.475 ± 0.057 (4)	0.666 ± 0.070 (4)	81.42 ± 0.61 (8)	1.84
Goosefish	0.319 ± 0.047 (5)	---	85.14 ± 0.53 (5)	--
Dogfish	0.393 ± 0.081 (4)	0.538 ± 0.044 (3)	75.89 ± 0.23 (5)	1.77
Trout* (Fresh Water)	0.824 ± 0.032	1.012 ± 0.034	77.75 ± 0.73	1.45

( ) number of corneas

* Edelhauser Exp. Eye Res 19:317, 1974.

trout and rabbit corneas. Of particular importance is the increased corneal water (81-85%) in the marine teleost corneas in comparison to the dogfish (76%) a non-swelling cornea (Smelser, Invest. Ophth. 1:11, 1962). This increased water content is probably related to the lack of complete stromal fusion that occu in the cornea of these species.

Measurement of HMS activity in the sculpin cornea is shown in Table 2. For whole cornea there is a preferential usage of the C_1 labeled glucose (indicative of HMS activity) which also occurs in the outer and inner cornea. The HMS activity in the component layers of the buphthalmic rabbit cornea are listed i Table 3. Although these corneas have been under the progressive stress of an elevated IOP (30-40 mm Hg) for a three month period, no increase HMS activity in either the epithelium or endothelium occurs; howeve increased HMS activity was measured in the stroma, relative to the control tissue. This may be related t a marked increase in stromal cell activity in reeponse to the increased pressure and corneal diameter (Van Horn et al., Exp. Eye Res. 25:171, 1977).

The results of this study indicate that the QO_2's of marine teleost and dogfish corneas are less tha FW teleost corneas, and that the total corneal water content of the marine teleost is greater than that o the dogfish, FW teleost, or rabbit. HMS activity is present in the sculpin cornea with measurable amount in both the inner and outer cornea. Buphthalmic stage 1 corneal disease does not cause an increase in HM activity in the corneal epithelium or endothelium but does in the stroma.

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Table 2

Mean C_1 CPM and C_6 CPM from ^{14}C-Glucose, and C_1/C_6 ratios for the Sculpin cornea

	Mean CPM per cornea		Ratio
	$^{14}C_1$	$^{14}C_6$	C_1/C_6
Whole Cornea (3)	383	90	4.65 ± 1.10
Outer Cornea (2)	259	60	6.26 ± 2.07
Inner Cornea (3)	357	45	9.33 ± 3.49

( ) Number of Samples

Table 3

Mean C_1 CPM and C_6 CPM from ^{14}C-Glucose, and C_1/C_6 ratios fro the component layers of the cornea of control and Buphthalmic Rabbits

	Control*			Buphthalmic**		
	mean CPM Per cornea		Ratio	mean CPM Per cornea		Ratio
	$^{14}C_1$	$^{14}C_6$	C_1/C_6	$^{14}C_1$	$^{14}C_6$	C_1/C_6
Epithelium	417	84	6.48 ± 2.6 (3)	650	127	6.82 ± 1.44 (4)
Endothelium	266	178	2.76 ± 1.07 (4)	150	93	2.10 ± 0.57 (4)
Stroma	140	38	4.34 ± 1.44 (4)	162	17	12.73 ± 4.58 (3)

( ) Number of Samples

* No corneal pathology, corneal diameter 14.5 mm

** Slight (+1) corneal clouding, corneal diameter 16.0 mm